



ASSESSING THE IMPACTS ON AQUATIC ORGANISMS
EXPOSED TO EMERGING CONTAMINANTS IN WASTEWATER DISCHARGES

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RESEARCH BACKGROUND

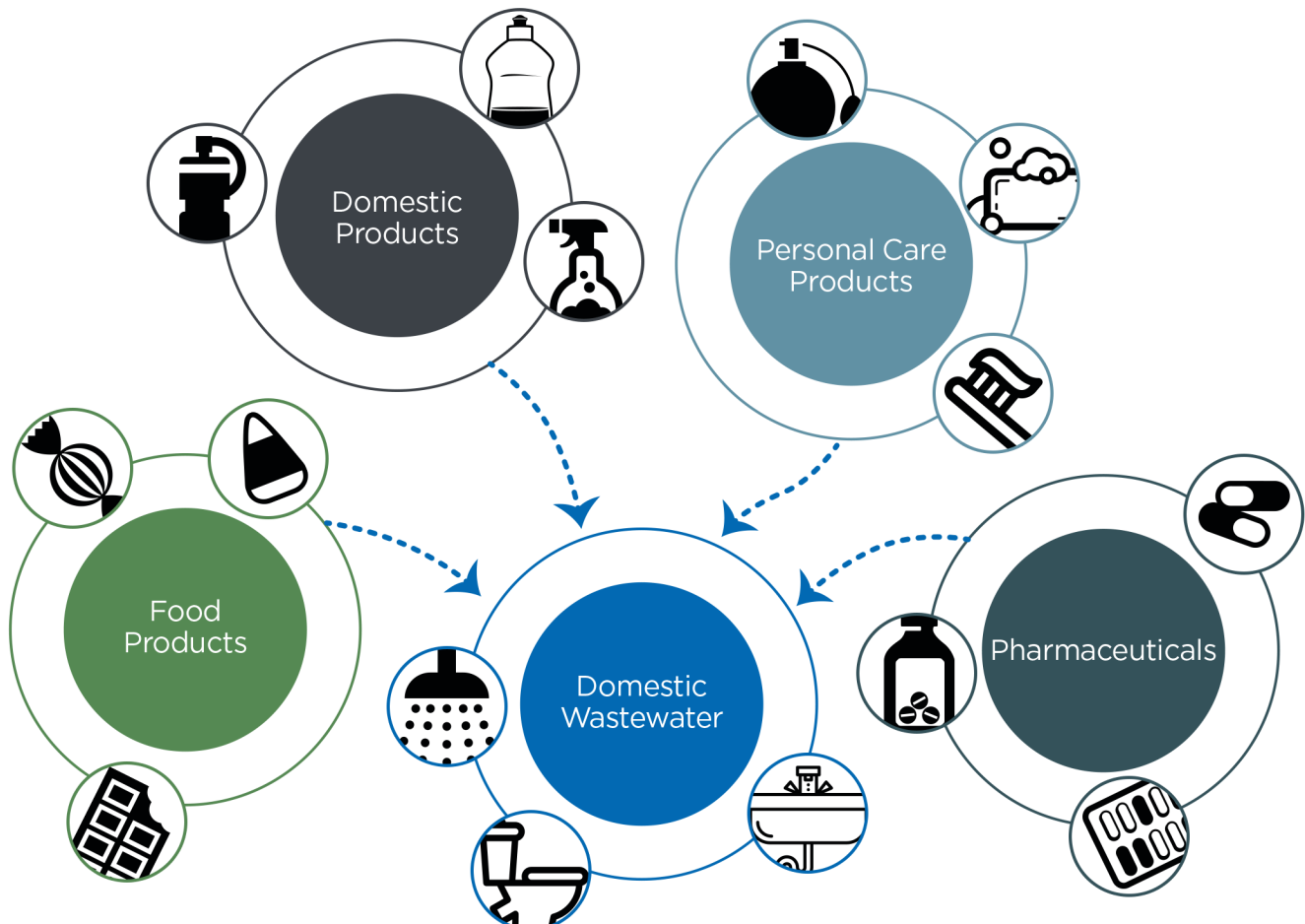
Raw domestic wastewater or sewage contains a mix of various chemicals from products that are part of our everyday life, such as pharmaceuticals, personal care products, low sugar foods, domestic products, illicit drugs and natural hormones (Figure 1). Scientific studies have proven that these chemicals are not efficiently removed by conventional technologies in wastewater treatment plants (WWTPs), but instead are released into rivers and lakes in Canada. These substances are known as Contaminants of Emerging Concern (CECs).

Previous research has indicated that aquatic organisms collected from waters contaminated by these chemicals have shown adverse biological effects, including changes in hormone levels, reduced immune function and intersex in the male sex organ. Research has also shown that some of these CECs accumulate in the tissues and blood of fish. To date, research has not directly linked exposure to CECs from wastewater discharge to adverse biological effects in aquatic organisms.

Although CECs may have effects on both aquatic organisms and drinking water quality, there are not yet any regulatory limits on their use. Municipalities are looking for methods that they can use to determine whether CECs released from their WWTPs are causing adverse effects.

The team collected wild fish, caged freshwater mussels and/or fish in Canadian rivers downstream of wastewater discharge and compared them to the condition of fish and mussels caged at upstream (uncontaminated) locations. In addition, the team collected fish from a lake that had been experimentally dosed with a synthetic estrogen to see if fish populations were recovering from this historical exposure.

Figure 1: Sources of Contaminants of Emerging Concern (CEC)



RESEARCH METHODS

The team used three approaches for this study:

1. Rainbow darter and greenside darter species were collected in the spring and fall of 2011 and 2012 from sites impacted by wastewater discharges in the Grand River (Ontario). Fish were examined for evidence of gonadal intersex and other indicators of exposure to CECs.
2. Freshwater mussels and fathead minnows were caged at locations upstream and downstream of wastewater discharges to evaluate whether there were any changes to their biological functions. The three study locations were Grand River, Ontario (upstream and downstream of the Doon WWTP), the St. Lawrence River (upstream and downstream of the Montreal WWTP) and the North Saskatchewan River (upstream and downstream of the Edmonton Gold Bar WWTP).
3. Fathead minnows and pearl dace were collected from Lake 260 at the Experimental Lakes Area near Kenora, Ontario, to evaluate biological functions in these fish species after exposure to the synthetic estrogen ethinylestradiol (EE2) was stopped.

RESEARCH FINDINGS

INTERSEX IN DARTERS COLLECTED IN THE GRAND RIVER (ONTARIO)

Among rainbow and greenside darters collected in the spring and fall of 2011 and 2012 from the Grand River, a high proportion (above 70%) of the male fish collected at locations downstream of WWTPs showed intersex of the gonad, as indicated by the presence of mature eggs in the testis (see Figure 2). The team's previous studies with aquarium fish have shown that this intersex condition can be caused by exposure to CECs from wastewater, including chemicals that mimic female estrogen hormones and chemicals that block the receptor for the male androgen.



Figure 2: Eggs in the testis of a male rainbow darter, as shown grossly (left) and in a histological section (right). The inset is an illustration of a male darter in breeding condition.

IMPACTS ON FORAGE FISH EXPOSED TO EE2 AT THE EXPERIMENTAL LAKES AREA (ONTARIO)

The synthetic estrogen in the birth control pill EE2 has been detected in wastewater discharges in Canada and in other countries. EE2 was continuously added to a lake at the Experimental Lakes Area (ELA) to understand whether this potent estrogen could cause biological and population-level responses in fish and other aquatic organisms. Many of the male fathead minnows developed gonadal intersex, and the population was almost eliminated from the lake as a result of poor reproductive success. Pearl dace and lake trout were also affected, although to a lesser extent.

In our study, we monitored these affected fish to see if they showed signs of recovery after EE2 exposure was stopped. Our studies showed that there was total recovery of fathead minnow populations and other fish species over the three-year period. This recovery to conditions that existed in the lake before EE2 was added show that investments to improve wastewater treatment to reduce CEC discharges will have benefits in terms of protecting aquatic life.

BIOMARKERS OF EXPOSURE IN CAGED FRESHWATER MUSSELS AND FATHEAD MINNOWS

Freshwater mussels and fathead minnow fish were caged at sites upstream and downstream of WWTP discharges in three Canadian rivers during the late summers of 2010 (St. Lawrence River), 2011 (Grand River) and 2012 (North Saskatchewan River). Analysis of CECs accumulated in passive samplers deployed in the rivers at the caging sites showed that concentrations of pharmaceuticals, personal care products and hormones were elevated at the downstream sites, as compared to the upstream sites. The combined results from the caging experiments in the three rivers demonstrated that there were biological responses in mussels and fathead minnows exposed to the wastewater plumes, and some of these responses could be attributed to exposure to CECs. Caged mussels and fish showed evidence of stress responses, and mussels showed enhanced function of their immune system. These responses could result from exposure to the sewage plume and may not be specific to CECs. However, the appearance of egg yolk protein (i.e. vitellogenin) in male fathead minnows caged at sites near the wastewater discharge is a clear sign of exposure to environmental estrogens.

An increase in the activity of liver enzymes responsible for removing toxic chemicals from the body in fish (i.e. CYP enzymes) also indicates exposure to pharmaceuticals and other contaminants in wastewater. These biomarker responses are relatively simple, yet sensitive methods that could be used by municipalities to assess adverse biological responses in aquatic organisms exposed to discharges from WWTPs.

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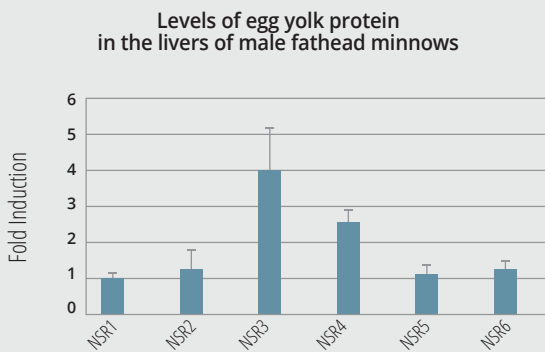


Figure 3: Levels of egg yolk protein (i.e. Vitellogenin induction) in the livers of male fathead minnows caged in the North Saskatchewan River for four weeks, at sites upstream (NSR1 and NSR2) and downstream (NSR3, NSR4, NSR5, NSR6) of the discharge from the Gold Bar WWTP.



Figure 4: Caging study of mussels in Canadian rivers

RESEARCH APPLICATION

Among Canadian municipalities, there is concern about the possible impacts on the aquatic environment from discharges of CECs in domestic wastewater. However, there is some uncertainty about whether these impacts are large enough to warrant investment in technologies that will remove these contaminants from the wastewater stream. In addition, municipalities question whether dilution from large river systems is sufficient to reduce the impacts on aquatic organisms.

This research has clearly demonstrated that WWTPs are the source of these contaminants in receiving waters. Municipalities can use this evidence when making decisions about wastewater treatment technologies and the subsequent effects on aquatic life.

In the Grand River, intersex of the gonad was observed in male darters collected at sites downstream of WWTP discharges. There were changes in biological function in fathead minnows and freshwater mussels caged downstream of WWTPs in three Canadian rivers with widely different hydrological regimes had effects on aquatic organisms. This research has clearly demonstrated that WWTPs are the source of these contaminants in receiving waters. Municipalities can use this evidence when making decisions about wastewater treatment technologies and the subsequent effects on aquatic life.

Specific biomarkers have been identified to assess changes in aquatic life that can be attributed to exposure to CECs. These biomarkers are a practical method that can be used by municipalities. Importantly, the post-exposure recovery of fathead minnow populations at the ELA lake dosed with EE2 indicates that investments in technologies to remove CECs from wastewater will benefit the health of aquatic organisms.

The information gathered from this research will assist in developing new policies to control and monitor CEC levels in WWTP's discharge and thereby reduce the harmful impacts on aquatic ecosystem.

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